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Original article

Antifungal susceptibilities of *Candida* species isolated from urine culture

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ABSTRACT

Candida spp. are the most common opportunistic mycosis worldwide. Although *Candida albicans* is the most common cause of urinary tract infections, the frequency of non-albicans *Candida* species is increasing with common use of antifungal in the prophylaxis and treatment. This may lead to difficulties in treatment. Antifungal tests should be applied with identification of species for effective treatment. In this study, identification of *Candida* species isolated from urine culture and investigation of susceptibility of these strains to amphotericin B, flucytosine, fluconazole, voriconazole was aimed. In this study, 58 *Candida* strains isolated from urine cultures at Osmaniye State Hospital between January 2012 and April 2013 were included. Urine culture and antifungal susceptibility tests were applied. Incidence rate of *Candida* spp. was determined as *C. albicans* (56.9%), *Candida glabrata* (20.6%), *Candida tropicalis* (10.3%), *Candida parapsilosis* (7%), *Candida krusei* (3.4%), *Candida kefyr* (1.8%). Most of the isolates were susceptible to amphotericin B, flucytosine, fluconazole, voriconazole. Twenty three (39.7%) *Candida* strains were isolated from internal medical branches and Intensive Care Unit and 12 (20.6%) from the Surgical Medical Branches. *C. albicans* and *C. glabrata* species were isolated most frequently as a candiduria factor in this hospital between January 2012 and April 2013. The analysis of antifungal susceptibility profile shows no significant resistance to antifungals.

1. Introduction

Urinary tract infections (UTI) are the most common nosocomial infections. Although UTI are usually created by bacteria, a broad variety of fungi, the majority of UTI are caused by *Candida* spp. but *Cryptococcus* and *Aspergillus* spp. are also prevalent [1,2]. *Candida* are opportunistic pathogens commonly found in nature. *Candida* species constitutes about 10–15% of nosocomial UTI [3,4]. UTI caused by *Candida* species is increasing rapid in recent years depending on the surgical and medical applications. Especially diabetes mellitus, urinary system defects, chronic renal failure, neutopenia, use of broad-spectrum antibacterial drugs, corticosteroids, immunosuppressive treatment and long-term urinary catheterization play important role in this rise [5,6]. In recent years, frequent use of antifungal prophylaxis and treatment results in infections with resistant non-albicans *Candida* species to antifungals [7].

Importance of antifungal susceptibility testing has increased to prevent or to minimize these problems. In this study, it was aimed to know the prevalence of *Candida* species that causing UTI and their antifungal susceptibility pattern.

2. Materials and methods

In this study, 58 *Candida* strains isolated from urine cultures of hospitalized patients in Osmaniye State Hospital Microbiology Laboratory between January 2012 and April 2013. Male and female patients were considered for our study. Only in patients who presented with signs and symptoms of urinary tract infections were included. Pure growth of yeast isolates with significant colony count was included in the study. The urine samples, where *Candida* species was isolated in the absence of pyuria, *Candida* with colony

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count ≤ 1000 CFU/ml and mixed growth (polymicrobial growth) were excluded from analysis.

A total of 58 non-duplicate *Candida* isolates were collected from patients who presented for various medical and surgical conditions. Their urine samples were sent to the microbiology laboratory for culture and antifungal susceptibility testing. The urine samples were collected in a sterile leak proof container with screw capped lids and transported immediately to microbiology laboratory. Urine wet mount examination was done to look for the presence of pus cells, red blood cells, casts, crystals or any bacterial or fungal elements. The urine samples were inoculated on blood agar (Oxoid, UK), EMB agar (Oxoid, UK) and Sabouraud's dextrose agar (SDA) (Oxoid, UK) by calibrated wire loop technique delivering 0.001 ml of urine as per standard protocol for urine culture. The culture plates were incubated aerobically at 37 °C for 24–48 h. *Candida* species isolated on culture plates with colony count > 10,000 CFU/ml were considered significant. The *Candida* isolates were further speciated by Gram stain, germ tube test and chlamydospor formation on corn meal agar (Oxoid, UK). Gram stained smears showed appearance of fungus as yeast budding. On corn meal agar the suspected *Candida* growth showed the formation of large, highly refractive, thick walled terminal chlamydospores. Germ tubes were seen as long tube like projections extending from the yeast cells on human serum inoculated with suspected strain of *Candida*. Further confirmation of identification and antifungal susceptibility testing was done for 58 *Candida* isolates using VITEK 2 Compact Sistem (BioMerieux, France). The antifungals which were tested included fluconazole, voriconazole, flucytosine and amphotericin B according to standard instruction from manufacturers [8]. Measurement of minimal inhibitory concentration (MIC) values of values of fluconazole, voriconazole, flucytosine and amphotericin B was determined according to the CLSI standard M27-A3 [9]. According to CLSI criteria *Candida krusei* strains are considered naturally resistant to fluconazole, all of these were considered resistant to fluconazole in this study. MICs for fluconazole ≤ 8 were considered susceptible (S), 16–32 μg/ml were dose-dependent susceptible (S-DD/I) and ≥64 μg/ml were considered resistant (R). MIC values of voriconazole ≤ 1 μg/ml were considered susceptible, 2 were dose-dependent susceptible (S-DD/I) and ≥4 were considered resistant. MIC values for flucytosine, ≤4 were considered susceptible (S), 8–16 μg/ml were dose-dependent susceptible (S-DD/I) and ≥32 μg/ml were considered resistant (R). MIC values for amphotericin B ≤ 1 (S), 2 (I), ≥4 (R) μg/mL were considered. *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *C. krusei* ATCC 6285, *Candida tropicalis* ATCC 750 were used as a standard strain.

2.1. Statistical analysis

The descriptive statistics was used to characterize the study group. Fischer's exact test was used for comparing the difference between the two groups. p value of <0.05 was considered as statistically significant.

3. Results

In this study, candiduria was more common in females 38 (65.5%), than in males 20 (34.5%) but not statistically significant ($p > 0.05$) (Table 1). Age range of patients who were positive for *Candida* spp. was from 19 till 91 years. Mean age was 67.1 ± 18.4 for all patients. Mean age was 69 ± 22.3 for male. Mean age was 66.1 ± 16.3 for female. The highest isolation rates of *Candida* among uropathogens were found in age group above 60 years. Distribution of species, clinics and antibiotic susceptibilities are shown in Tables 2 and 3. *C. albicans* (56.9%), were predominant compared to non albicans *Candida* species. There was no case that isolated the multiple species. Incidence rate of *Candida* spp. was determined as *Candida glabrata* (20.6%), *C. tropicalis* (10.3%), *C. parapsilosis* (7%), *C. krusei* (3.4%), *Candida kefyr* (1.8%). From Internal Medical Branches and the Intensive Care Unit (ICU) 23 (39.7%), from Surgical Medical Branches 12 (20.6%) *Candida* strains were isolated. Amphotericin B was found the most effective antifungal agents. Any other significant resistance was not observed against to other antifungals. One strains of *C. glabrata* were intermediate resistant. Resistance to fluconazole was a little bit higher than flucytosine, voriconazole; one *C. albicans*, two *C. krusei* strains were in the resistance zone of susceptibility and two *C. glabrata* were in the intermediate zone of susceptibility (*C. krusei* is considered intrinsically resistant to fluconazole). Resistance to flucytosine was seen in one *C. tropicalis* strains, one *C. glabrata* were in the intermediate zone of susceptibility. One *C. albicans*, one *C. krusei* strains were found in the resistance zone of susceptibility.

Table 1
Age and gender distribution of *Candida* isolates.

Age group	Male, *n* (%)	Female, *n* (%)	*p*[a]
19–60	6 (43)	8 (57)	$p > 0.05$
>60	14 (32)	30 (68)	$p > 0.05$
Total	20 (35)	38 (65)	$p > 0.05$

[a] Fischer exact test: $p < 0.05$ was considered as statistically significant.

Table 2
Distribution of *Candida* isolates according to the clinics.

Species (*n*)	Internal medical branches, *n*	Surgical medical branches, *n*	Intensive care unit (ICU), *n*
C. albicans (33)	12	8	13
C. glabrata (12)	7	2	3
C. tropicalis (6)	–	–	6
C. parapsilosis (4)	2	1	1
C. krusei (2)	1	1	–
C. kefyr (1)	1	–	–
Total (58)	23 (39.7)	12 (20.6)	23 (39.7)

4. Discussion

C. albicans was the most frequently isolated *Candida* species from urine cultures, *C. glabrata* was the second most frequent species in this study. Most of the isolates were susceptible to amphotericin B. A total of three isolates resistant to fluconazole; two of them are *C. krusei* strains that considered naturally resistant. Two strains were resistant to voriconazole, one isolate was found to be resistant to flucytosine.

Urinary tract infections are the most common bacterial infections in hospitalized patients. Usually bacteria are common factor, fungal etiology is identified among them as 10% and *Candida* species are located in the first row [3,10]. Frequency of UTI caused by *Candida* species has increased steadily since the 1980s. Longer duration of hospitalization, urinary tract abnormalities, immune-compromised patients, long-term broad-spectrum antibiotic use, prophylaxis with antifungals are major risk factors for causes of UTI. Candiduria is a major cause of morbidity and mortality in those no receiving appropriate diagnosis and treatment [11].

C. albicans is the main agent accounting for 40–65% of the fungi isolated from *Candida* colonization cases. However, there is an increase in the incidence of other species, and the urinary tract now is more frequently colonized by non-albicans species notably *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei* along with other yeast, like *Trichosporon* species [12]. But some studies have shown